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P01/7700 25.00 - 9821170.9

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
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1. Your reference — P21896/CPA/RMC

2. Patent application number *(The Patent Office will fill in this part)* — **9821170.9**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

University of Ulster at Jordanstown
Newtownabbey
CO ANTRIM
BT37 0QB

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

5965918003

4. Title of the invention — "Marker"

5. Name of your agent *(if you have one)*

Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

373 Scotland Street
GLASGOW
G5 8QA

Patents ADP number *(if you know it)* — 1198013

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

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9. Enter the number of sheets for any following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	6
Claim(s)	N/A
Abstract	
Drawing(s)	

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10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 30 September 1998

Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom

Roisin McNally, 0141 307 8400

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Notes

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"Marker"

The present invention relates to a method of diagnosis of bladder cancer. More particularly the invention relates to an accessible marker for bladder cancer.

Transitional cell carcinoma of the bladder accounts for 1% of all cancers and is the fifth most common malignancy in the over 60s in industrialised parts of the world (Russell et al., 1988; Gleave et al., 1993). Eighty percent of all bladder TCC is superficial at presentation; the remaining 20% is muscle invasive and 50% of patients in this category die despite treatment (Simoneau and Jones, 1994). Of those patients initially presenting with superficial tumours, 50 to 70% have recurrences within two years. These recurrences are usually superficial although 10 to 20% progress to the muscle invasive form (Parmer et al., 1989; Fradet, 1992; Harland, 1994).

The high frequency of recurrent TCCB and the increase in disease status in a proportion of patients means that lifetime follow-up using cystoscopy and urinary cytology is essential. The standard procedure is an initial check cystoscopy three months after disease

presentation; if this is clear cystoscopy should then be carried out every six months, for one to two years and then annually thereafter with a flexible cystoscope. At present the recurrence rate of TCCB means that annual lifetime cystoscopies should be carried out for all stabilised patients.

Cystoscopy involves insertion of a cystoscope into the bladder via the urethra to allow visualisation of the tumour using fibre optics. It confirms clinically and pathologically the presence of tumour within the bladder and allows a morphological description (Hossan and Striegal 1993). However it has the disadvantages of being an invasive, uncomfortable procedure. The frequent recurrences of TCCB mean that patients must undergo lifetime follow-up using cystoscopy; this results in the further disadvantage of a large expenditure by the health service.

Urine cytology is used for the detection of recurrent bladder TCC and although it offers the advantages of being a non-invasive, inexpensive, easily accessible procedure (Zein and Milad, 1991), it has a poor sensitivity, especially at lower stages and grades of disease. The result is false positive and negative findings with reported sensitivities ranging from 37.9% (Miyanaga eta al., 1997) to 64% (Martins et al., 1997).

Numerous studies have been carried out to find the ideal bladder cancer marker. However, none are adequately sensitive or specific enough to fulfil a diagnostic role at present. The most successful to date appears to be the Bard BTA, STAT and TRAK tests with overall sensitives of 55% (Bard promotional information), 72% (Leyh et al., 1997) and 88% (Bard promotional information) respectively.

Bladder cancer is a frequently recurring disease; patients require lifetime monitoring using cystoscopy and urinary cytology. Cystoscopy is an invasive technique and urinary cytology while non-invasive has a low sensitivity.

It is an aim of the present invention to replace these two procedures with a sensitive, non-invasive urinary test which would allow detection of first presentation and recurrent bladder cancer.

The invention relates to a 37KDa epidermal growth factor receptor (EGFR) fragment in the urine of patients with transitional cell carcinoma of the bladder (TCCB).

According to the present invention there is provides a marker for bladder cancer, the marker comprising a 37KDa EGFR fragment which is detectable in urine.

The invention provides a test for the presence of a 37KDa EGFR fragment in urine, the test comprising a western blot assay.

Alternatively the test may comprise an immunochromatographic assay, an ELISA test, latex agglutination or radioimmunoassay.

The invention further prodies a method of diagnosing bladder cancer, the method comprising the steps of reacting a urine sample from an individual to be tested with means to detect a 37KDa EGFR fragment and analysing results.

Preferably the test is in the form of a dip stick.

A 37KDa EGFR fragment has been detected in urine from patients with bladder cancer. First morning urine samples were collected from 24 TCC patients, 6 patients who had bladder cancer previously but who were no disease free and 13 healthy volunteers. 10mls of urine from each was freeze dried and the powdered residue reconstituted in Laemmli lysis buffer. After heating at 110°C for 20 minutes, all samples were stored at -70°C until required for analysis. Samples were then probed with the Ab4 EGFR antibody (Oncogene Sciences) to the internal domain of the receptor by western blot analysis.

A 37KDa fragment was detected in 88% (21/24) of TCC patients, 66% (4/6) of disease free patients and 7% (1/13) of healthy volunteer urine samples. There was an overall significant association between detection of the 37KDa fragment and presence of bladder cancer. Although four out of six patients who were disease free tested positively, two had benign tumours and two had bladder inflammation at the time the urine sample was taken. This 37KDa fragment therefore appears to be of diagnostic importance. It has a much higher sensitivity than urinary cytology and the Bard BTA and STAT tests, and it appears to be comparable to the Bard TRAK test.

The high frequency of recurrent TCC in the bladder and the progression to a more malignant phenotype in a proportion of patients means that lifetime follow-up using cystoscopy and urinary cytology is essential. Cystoscopy is an evasive procedure and urinary cytology while non-invasive is relatively insensitive. At present the Bard BTA and STAT tests are the only commercially available detectors for bladder cancer. Their sensitivity means that at best they will only act

in conjunction with cystoscopy. The Bard TRAK test while more sensitive has yet to be marketed and in fact the results from the present study indicate that the 37KDa EGFR fragment is at least comparable. Further work is required to investigate the significance of this fragment in the detection of first presentation and recurrent bladder TCC and to determine whether making it into a quantitive test will offer some insight into prognosis. Appropriate applications are detailed below.

The 37KDa EGFR fragment may be used as a detector for first presentation bladder and recurrent bladder TCC. Detection of the 37KDa EGFR fragment may be carried out by other methods of investigation as well as western blot analysis. These methods may include immunochromatography, ELISA, latex agglutination or radioimmunoassay. There is currently available a one-step immunochromatographic assay which qualitatively detects bladder tumour antigen in urine in five minutes. Detection of the 37KDa EGFR fragment may be detected by a similar method. Patient urine would be added to the small chamber where it mixes with a colloidal gold-conjugated antibody. If the 37KDa fragment is present, a 37KDa fragment conjugate complex would form. The reaction mixture would flow through the membrane which contains zones of immobilised capture antibodies. In the test zone, the 37KDa fragment conjugate complexes would be captured by a second antigen-specific antibody, forming a visible line. If the 37KDa fragment is not present in the urine, no visible line would form.

Also or alternatively a dip-stick test may be developed. This may require using methods such as latex agglutination, immunochromatogrphy, ELISA and

radioimmunoassay.

Bladder cancer prognosis has been correlated with a number of factors, the single most important of which is depth of invasion of the bladder wall (Gospodarowicz, 1995); this is followed by grade of tumour (Heney et al., 1983). Other less important factors which influence patient outcome include tumour size (Gospodarowicz, 1995), age of patient at diagnosis (Fitzpatrick and Reda, 1986) and health status (Thrasher et al, 1994). None of these factors can predict prognosis in 100% of patients and so the 37KDa fragment may have some use prognostically. The EGFR fragment may be detected quantitatively using densitometry following western blot analysis and used to predict whether increased levels indicate a better or worse prognosis. Other quantitative methods may be developed to allow easier performance e.g. ELISA or radioimmunoassay techniques.

EGF and EGFR have been implicated in the pathogenesis of solid tumours such as those of the breast. This simple test developed for urine of patients with suspected TCCb might also be used to identify the diagnostic prognostic role of serum EGFR in other tumour types.